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MECHANISMS AND TREATMENT OF LUNG LESIONS AND ASSOCIATED SURFACT--ETC(U)
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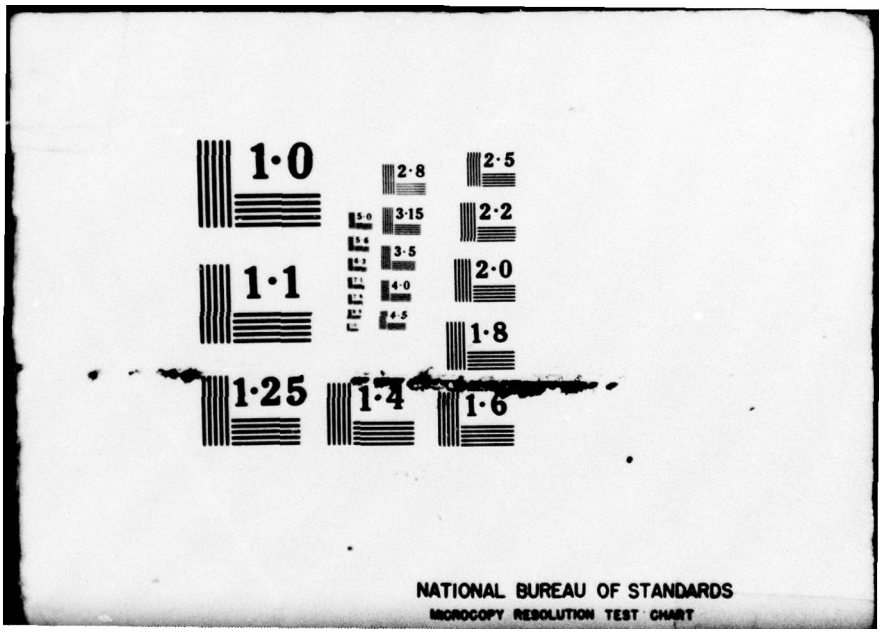
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REPORT NO. 18

Mechanisms and Treatment of Lung Lesions and Associated Surfactant Damage in Shock.

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A. Antibiotic coverage in severe gram negative septicemia in conjunction with early antishock treatment.

A series of mortality experiments were performed which demonstrated that antibiotic coverage of lethal gram negative bacteremia is effective only in conjunction with early antishock treatment with steroids. Since mortality experiments cannot be realistically performed in primates rats were used for the above studies because, like primates, they are resistant to generalized Shwartzman reaction. Further studies, summarized in this report, indicated that lethal gram negative bacteremia in rats results in sequential structural alterations of lung and liver which are similar to those observed in the primate experiments. In addition, experiments using the rat model were found to be exceptionally reproducible for pharmacologic experiments designed to evaluate the effects of therapeutic regimens on lethal bacteremia. Therefore, in our future research effort we plan to use extensively the rat model before appropriate experiments in primates are performed. This approach has already considerably accelerated our research activity in this field and also enabled us to be more selective and effective in designing and performing elaborate experiments in primates, which are not only extremely time consuming but also very expensive.

We have completed a series of morphologic and biochemical studies on the effects of glucocorticoid and antibiotic treatment on lethal gram negative bacteremia in rats. In these experiments four groups of male rats, weighing 300 $\pm$ 20 grams, received I.V. 2.5×10^9 live E. coli: group A received this bolus without additional treatment; group B received I.M. gentamicin sulfate, 5 mg/kg, immediately following bolus E. coli infusion; group C received I.V. methylprednisolone sodium succinate, 40 mg/kg, immediately following E. coli injection; group D were treated as rats of group C but in addition they received gentamicin I.M., 5 mg/kg, immediately after E. coli injection. Controls included normal rats which received saline infusion only. The survival rates of the four test groups at 10 and 20 hours were 25% and 4% for A; 44% and 28% for B; 94% and 70% for C; 98% and 98% for D groups, respectively. Rats from each group were killed at 2, 6 and 10 hours and tissues from lung and liver were immediately placed in fixative for electron microscopy. Tissues from all major organs were placed in formalin for light microscopy. In addition blood samples were obtained prior to sacrifice from each rat through intracardial puncture for biochemical studies.

In group A pathological changes of the lung were essentially similar to those previously described in septic shock in primates. The liver also revealed similar qualitative changes as those found in the primate model. These changes included intravascular sequestration of leukocytes; fibrinous deposits in sinusoids and Disse's space; extensive disruption of the sinusoidal endothelial lining; and progressive hepatocellular injury leading to severe centrilobular necrosis. These sequential reactions were associated with rapid hypoglycemia which was severe in animals killed at 10 hours. In addition there was progressive elevation of serum LDH, GOT, GPT and bilirubin. The severity of the above biochemical changes appeared to be proportional to the degree of structural liver damage. Histologic evaluation of various organs demonstrated progressive bacterial microembolization, especially prominent in kidneys (glomeruli), lungs and heart.

Rats of group B revealed similar biochemical and morphologic changes as animals of group A with the exception that, due to antibiotic treatment, bacterial microemboli were not observed in organs of group B rats. By contrast, animals treated with steroids (especially those of group D) demonstrated a striking improvement of the serum biochemical profiles tested as well as marked reduction in the severity of sinusoidal reactions and associated progressive liver damage. It should be emphasized that extensive bacterial microembolizations, having a distribution similar to that seen in group A rats, was found in steroid-treated rats of group C which did not receive the antibiotic.

These findings suggest: (a) host reactions leading to lethal gram negative shock are determined by the rate and level of endotoxin release rather than level of bacteremia or extent of bacterial microembolization; (b) antibiotic treatment alone has no effect on lethality and host reactions in potentially fatal gram negative bacteremia; (c) protection of the host against the effects of antibiotic-induced endotoxin release is a rational approach to the treatment of lethal gram-negative shock; (d) the protective effect of glucocorticoids on liver microcirculation represents a basic mechanism in attenuating lethality of endotoxin shock.

B. Implementation of plans to organize a functional laboratory for vasoactive substances.

Since bradykinin and components of kinin generating system are believed to play a basic role in the pathophysiology of septic shock, we have devoted considerable effort to develop a functional laboratory for the investigation of the kinin generating system in the primate septic shock model. The following are the main components which are under investigation in our laboratories: (a) prekallikrein; (b) kallikrein; (c) kininogen; (d) kinins; (e) kininase.

In addition, an attempt will be made to correlate the coagulapathy observed in the primate shock models with the alteration in the kinin generating system. To our knowledge similar studies have not been conducted in primates and the information will prove extremely valuable not only to our research but to other investigators who are employing primate models in this type of research.

We are now implementing special blood collecting procedures to preserve the kinins (use of kininase inhibitors). In addition, we are in a process of developing a minicolumn procedure to obtain kininogen. The total kininogen assay will then be performed by liberating kinins from these protein concentrates (enzymatic) or by radial immunodiffusion method. Our laboratory is now equipped to measure kinin levels using isolated tissue preparations such as the guinea pig ileum and rat uterus preparations. We have also standardized methodologies to differentiate other autocoids from kinins by enzymatic and blocking methods. We are further planning to employ radioimmunoassay (RID) to determine kallikrein, kininogen and bradykinin in the plasma and tissue extracts of the experimental animal.

I should emphasize that it will take some time before we acquire adequate expertise in this exciting but also very complex field. We strongly feel, however, that the investment will be worthwhile since

such studies will enable us to correlate in the primate shock models pulmonary blood-vascular reactions with changes in blood coagulation and kinin generating system.

Addendum: Studies Completed.

A. Purification of lung surfactant, see appended reprint #R1. The results of our study designed to investigate the nature of lipid-protein complexes of lung washing has led to the development of a simple and rapid density gradient centrifugation method for surfactant purification. The lipid composition of surfactant from normal rabbit lung washing isolated by this method compared quite favorably with surfactant isolated by much more elaborate and time consuming procedures. Moreover, surfactant purified from mixtures of lung washing and ^{3}H-palmitate labelled rabbit serum contained less than 3% of the phospholipid radioactivity. In addition, the surfactant band isolated from the above mixture had a phospholipid composition which was quite similar to that of surfactant from normal lung washing but it had a much higher protein content. However, a second gradient centrifugation removed 90% of this protein resulting in a surfactant fraction with a phospholipid to protein ratio similar to that of surfactant from normal lung washing. Therefore, the new purification method is capable of removing large proportion of both serum phospholipids and proteins from lung washing contaminated with serum, making this method uniquely suitable for the evaluation of both human and animal surfactant in various pathologic conditions of the lung. In addition this method can be readily applied to small samples such as tracheal aspirates, using as little as 1 ml of starting material for the isolation of surfactant. The method of density gradient centrifugation was also applied to residual lung tissue remaining after multiple alveolar lavages (x10) in an attempt to isolate intracellular surfactant. Following vigorous homogenization of the tissue, intended to permit complete recovery of the intracellular surfactant and not expected to yield intact lamellar bodies, and a low speed centrifugation (to remove connective tissue and cell debris), the lung homogenate was used to prepare gradients in the same way as lung washing. Following centrifugation, a band of particulate material was clearly visible at the same position in the tube as for lung washing surfactant. About 40% of the phospholipid of the lung homogenate was found in the band along with less than 1% of the protein. The surfactant fractions from the residual lung tissue were surface active but did not reduce the minimum surface tension to the very low values found with lung washing surfactant. Residual lung surfactant contained slightly more neutral lipids than did the surfactant isolated from lung washings, which may account for reduced ability of the intracellular surfactant to lower surface tension. Phospholipid analysis of the residual lung homogenate and the surfactant purified from it revealed that the amount of PC increased after density gradient centrifugation from 50% to 70% and the palmitic acid content of the PC increased from 43% to 57%. However, the residual lung surfactant purified in this manner has a phospholipid composition somewhat different from that of the surfactant from lung washing.

B. Characterization of lung washing proteins, see appended reprint #R2. A major problem in the study of proteins from lung washing and particularly of purified lung surfactant fractions is the presence of large quantities of lipids. Detergents or organic solvents, both of which may alter proteins, have previously been used for the separation of lipids and protein. We have applied a gentler technique, recently described for serum, to delipidate

lung washings and density gradient fractions from lung washings. Diisopropyl ether-butanol (6:4) is added in a 2:1 (v/v) ratio to aqueous sample of lung washings and following mixing for 30 min. most of the lipid is found in the organic phase while the protein remains in aqueous solution. Proteins from lung washings prepared in this manner were used to immunize a goat giving us an anti-lung washing protein serum. Immunoelectrophoretic methods as well as polyacrylamide gel electrophoresis (PAGE) were then used to characterize the proteins of lung washings and lung washing fractions. PAGE revealed that lung washings contain at least 7 protein bands when stained with Coomassie blue, one of which also stained with PAS. In SDS-PAGE, 12 peptides were observed and these had molecular weights ranging from 10,000 to over 300,000. A prominent glycoprotein was seen by PAS in the 300,000 molecular weight range. Two dimensional crossed immunoelectrophoresis (CIEP) of the lung protein against anti-lung washing protein serum revealed several antigen-antibody peaks, the largest of which was characteristic of albumin. The second most prominent antigen-antibody precipitate appears to be a non-serum antigen. Further immunoelectrophoretic studies indicated that the non-serum antigen is IgA, similar to findings reported by Colacicco, et al. Surfactant from normal lung washing, isolated by the density gradient method, consistently contains a small amount of protein. In order to determine if any of the lung washing proteins were specifically attached to the surfactant phospholipids, the protein composition of various density gradient fractions were compared. By CIEP, both surfactant and non-surfactant fractions were seen to contain the same antigens in apparently similar quantities. No individual protein was more concentrated in the surfactant fraction than in any other fraction. These results confirm our previous studies suggesting that the protein in isolated surfactant fractions is not part of a specific lipoprotein molecule, but results from non-specific trapping of protein in liposomes formed during aqueous lavage procedures. These studies, however, do not provide answers with respect to the role of proteins associated with the surfactant system.

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20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

A. Glucocorticoid and antibiotic effects on lethal gram negative bacteremia;

Survival rates and host responses were evaluated in four groups of male rats, weighing 300 ± 20 gm, which received IV 2.5 x 10^9 live E. coli. This bolus was given either without additional treatment (A) or prior to the following regimens: IM injection of gentamicin sulfate, 5 mg/kg (B); IV injection of methylprednisolone sodium succinate, 40 mg/kg (C); and gentamicin IM immediately after glucocorticoid treatment (D). Survival

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rates at 10 and 20 hours were 25% and 4% for A; 44% and 28% for B; 94% and 70% for C; 98% and 98% for D groups, respectively. In rats of groups A and B, killed at 2-10 hours, progressive liver changes included intravascular sequestration of rapidly degranulating leukocytes, fibrinous deposits as well as platelet aggregates in sinusoids and spaces of Disse, and extensive Kupffer cell disruption in association with diffuse glycogen depletion and severe midzonal necrosis. These alterations were accompanied by progressive hypoglycemia and elevations of serum enzymes, GPT, LDH and GOT. All above reactions were delayed and, with the exception of glycogen depletion, markedly reduced in rats of groups C and D. The results indicate that antibiotic coverage of lethal, gram-negative bacteremia is effective only in conjunction with early steroid treatment. The protective effect of glucocorticoids on liver microcirculation appears to be a basic mechanism in preventing the development of hepatocellular necrosis and associated major host responses thereby attenuating lethality of gram negative septic shock.

B. Purification of surfactant from lung washings and washings contaminated with blood constituents.

A rapid and simple method capable of purifying surfactant from rabbit alveolar washings and from washings contaminated with serum has been developed. The findings demonstrate that this purification method is capable of removing a large proportion of both serum phospholipids and proteins from lung washings contaminated with serum, making this method uniquely suitable for evaluation of surfactant in pathologic conditions of the lung.

C. Isolation and characterization of proteins associated with the lung surfactant system.

Rabbit lung washings and purified lung surfactant were delipidated without precipitation or loss of protein. This enabled effective study of the proteins by electrophoretic and immunoelectrophoretic techniques. The lung washings contained secretory immunoglobulin A and several serum proteins. The protein composition of purified lung surfactant was the same as the unfractionated lung washings confirming our previous study which indicated that there is no specific protein associated with surfactant phospholipids obtained by alveolar lavage with isotonic saline.